Scientific Abstract

Malignant gliomas are the most common primary brain tumors of humans, accounting for 30% of all central nervous system (CNS) tumors in adults. They two most common types are: (i) anaplastic astrocytoma and (ii) glioblastoma multiforme. Primary malignant brain tumors in the United States are estimated to occur at an incidence of 14.7 per 100,000 people, and 10,000 - 15,000 new cases are diagnosed annually (Levine et al.,1989). Multimodal approaches to treatment, such as surgery, radiation, and chemotherapy, have extended the median survival of patients with malignant gliomas from 14 weeks to one year; however, the five year survival rate for glioblastoma multiforme is still 5.5% or less. The disease is characterized by local tumor recurrence with relentless regrowth, causing neurologic dysfunction and ultimately death. Thus, new treatments for malignant gliomas are needed.

G207 is a modified HSV-1 virus that has desirable properties as a tumor-killing agent (Mineta et al., 1995). G207 was constructed by Martuza and colleagues by inserting the coding region of the E. coli lacZ gene into the UL39 gene of an HSV-1 mutant termed R3616 (Mineta et al., 1995). R3616, has a 1 kb deletion in both copies of the major neurovirulence factor gene, γ34.5 (Chou et al., 1990). The insertion of lacZ into the HSV UL39 gene destroys the UL39 gene, which normally encodes a protein termed ICP6. ICP6 is the large subunit of the HSV-1 ribonucleotide reductase, which is required by the virus for replication in non-dividing cells (Goldstein and Weller, 1988). Thus, G207 lacks the ability to make the neurovirulence factor γ34.5 and the viral ribonucleotide reductase. Instead, it can make β-galactosidase from the lacZ gene, which serves as a useful molecular marker. MediGene, Inc. has confirmed the DNA structure of G207 using Southern blot analysis with probes to lacZ, UL39, and γ34.5 sequences, and by sequencing of the manipulated junctions. G207 was tested for anti-tumor efficacy and safety in mice, and for safety in the *Aotus nancymae* monkey prior to use in clinical trials.

An open label, dose-escalating, phase I study of the intratumoral injection of G207 was completed in March 2000 in subjects with malignant glioma who failed conventional therapy [OBA # 9802-235 (MediGene Protocol NG1-001): “A Dose Escalating Phase 1 Study of the Treatment of Malignant Glioma with G207, A Genetically Engineered HSV-1”]. The primary objective of the study was to obtain safety information in small numbers of individuals (three subjects per group) who were treated with escalating doses of G207, and to identify a Maximum Tolerated Dose (MTD).

A total of 21 subjects, seven cohorts of three subjects had been enrolled. Subjects were treated at dose levels of 1×10^6, 1×10^7, 3×10^7, 1×10^8, 3×10^8, 1×10^9 or 3×10^9 pfu G207. Subjects in cohorts one to five received a single stereotactic injection of 100 μL. Subjects in cohort six received a single stereotactic injection of 300 μL and subjects in cohort seven received five stereotactic injections of 200 μL each.

G207 was generally well tolerated and safe in NGI-001, and no dose-limiting toxicity was observed. A total of 19 subjects (90.5%) died with two remaining alive to date. All deaths except one (radiation necrosis) had been attributed to progressive disease.

In a compassionate use study, one male subject with glioblastoma multiforme who had failed multiple conventional therapies was treated with G207 (MediGene Protocol NG1-002: "A Single Patient Compassionate Use Study of G207, a Genetically Engineered Herpes Simplex 1 Virus, in the Treatment of Glioblastoma Multiforme"). The subject received 1×10^9 pfu of G207 via multiple injections into the tumor bed at the time of re-resection. He tolerated the procedure well without neurological or other sequelae. The subject subsequently died from his disease.

MediGene, Inc. would like to continue to evaluate G207 as monotherapy in patients who have been diagnosed with recurrent malignant glioma of the brain. Protocol NG1-003 is a phase lb/II study, wherein up to 21 subjects will be enrolled in the phase lb portion and will receive doses of G207 that are higher than tested in the previous trial (OBA # 9802-235, MediGene Protocol NG1-001). The phase 1b portion of NG1-003 is a continuation of trial NG1-001. The study objectives are to determine the safety and tolerability of G207, and to ascertain whether or not G207 replicates within tumor tissue. In addition, the ELISPOT assay will be employed to assess cellular response to tumor as a potential surrogate marker for efficacy. The dose to be used in the phase II portion of NG1-003 will also be determined.

Subjects in the phase I portion of NGI-003 will receive G207 in a fractionated manner. Initially, 15% of the total dose will be injected into the tumor. Two days later the tumor will be resected and the remaining dose will be inoculated into the tumor bed at the time of resection. The following doses will be evaluated: 1×10^9, 3×10^9 and 1×10^{10} pfu. Subject status will be monitored by MRI, Karnofsky performance, neurologic examination and the presence of virus, in addition to other clinical assessments performed at specific study timepoints. Lastly, a MRI sub-study will examine the ability of MR spectroscopy to detect tumor progression and to differentiate radiation necrosis from HSV infection.

After three months of evaluation, subjects will be followed for safety and survival in a long-term protocol, NG1-004, for up to 12 months and, thereafter, by telephone. Results from the phase 1b portion will be reviewed, summarized and submitted to NIH in executive summary format prior to initiating the phase II portion of NGI-003 with new enrollees. The phase II will only commence provided that there are no safety or other concerns.

The design of the phase II portion of protocol NG1-003 is a classic NIH, two-stage study and will be multi-center in nature. The objectives are to assess the safety of G207 and subject survival at six months. Enrollment of up to 14 subjects is planned for stage one. Additional subjects will be enrolled (up to 30 subjects and 44 overall) in stage II if there is

a survival benefit for up to five subjects for six months or longer in stage one. Participants will receive a single dose of G207 at the dose determined from phase lb. G207 will be inoculated into the tumor bed at the time of resection. Again, subject status will be monitored by MRI, Karnofsky performance, neurologic examination and the presence of virus, in addition to other clinical assessments performed at specific study timepoints. After six months of evaluation, subjects will be followed for safety and survival in a long-term protocol, NG1-005, for up to 12 months and, thereafter, by telephone.

Literature Cited

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